

DNA Damage Induced by Gaseous Formaldehyde on Marrow Cells of Mice Tested by RAPD Assay

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Abstract—In order to explore distant-site genotoxic effect of gaseous formaldehyde on genetic material of marrow cells in mice. SPF-class Kun Ming male mice were exposed to gaseous formaldehyde for 72 hours in such concentration as $0.5\text{mg}/\text{m}^3$, $1.0\text{mg}/\text{m}^3$ and $3.0\text{mg}/\text{m}^3$. We applied the random amplified polymorphic DNA (RAPD) method to evaluate the genotoxic effect of the gaseous formaldehyde. The results of the RAPD profiles showed obvious differences between normal and exposed groups. The number of the varied bands increased gradually with the concentration of gaseous formaldehyde. Genomic template stability (GTS) analysis showed that with the increase of the gaseous formaldehyde concentration, the polymorphisms were 50%, 39.66%, 32.76% and the GTS were 50%, 60.34%, 67.24% respectively. These results suggest that gaseous formaldehyde has significant genotoxicity and damage effect on the genetic material in marrow cells in this concentration range. Meanwhile, the DNA polymorphisms detected by RAPD technique could be used as a biomarker for detecting genetic effects of formaldehyde stress on mice.

Keywords- formaldehyde, marrow cells, RAPD, DNA damage, Genotoxicity

I. INTRODUCTION

Formaldehyde is the most simple yet most reactive of all aldehydes, with the chemical formula CH_2O [1] and [2]. Given its economic importance and widespread use, many people are exposed to formaldehyde environmentally and occupationally. Occupational exposure involves not only individuals employed in the direct manufacture of formaldehyde and products containing it, but also those in industries utilizing these products, such as construction. Formaldehyde was long considered as a probable human carcinogen (Group 2A chemical) based on experimental animal studies and limited evidence of human carcinogenicity. However, the International Agency for Research on Cancer (IARC) reclassified formaldehyde as a human carcinogen (Group 1) in June 2004 based on “sufficient epidemiological evidence that formaldehyde causes nasopharyngeal cancer in humans”. The sufficient evidence comes from six major cohort studies of industrial workers and seven cases-control studies of nasopharyngeal cancer [3].

Some authors have argued that it is biologically implausible for formaldehyde to cause leukemia. Their primary arguments against the human leukemogenicity of formaldehyde are: (1) it is unlikely to reach the bone marrow and cause toxicity due to its highly reactive nature; (2) there is no evidence that it can damage the stem and progenitor cells, the target cells for

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leukemogenesis; and (3) there is no credible experimental animal model for formaldehyde-induced leukemia. Indeed, IARC itself concluded that “based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukemia in humans” and stated that “this is an area needing more research” [3, 4].

There is a need for scientists in public health, epidemiology and toxicology to generate new data on the question of biological plausibility and to work with national, international and regulatory agencies reviewing this controversial issue. This experiment took bone marrow cell of mice as study object, and make use of RAPD technology to discuss the DNA damage formaldehyde did to marrow cells of mice. So, we can go a step further to discuss if formaldehyde were connected with leucocytopenia. And provided a reference to study of formaldehyde and leucocytopenia in molecule level. From this experiment we anticipate to know toxicity of formaldehyde more allround. And it provided solid theory basis to build the system, which used to take precaution against organic, contaminates and control it.

II. MATERIALS AND METHODS

A. Experimental animal

Female SPF Kun Ming mice, five weeks old, body weight as $20\sim 25\text{g}$, were purchased from the Experimental Animal Center of Hubei.

B. Main Reagents and Apparatus

Random amplification primer, Proteinase K, RNase, Tap DNA polymerase, dNTPs, DNA-Marker were all purchased from Sangon. SDS were purchased from Merk. Trisbase and Agarose were purchased from Sigma.

Apparatus include 1800spectrophotometer (F-4500); Centrifuge (Eppendorf-5415R); DNA Engine (Bio-rad); Fluorescence spectrophotometer (RF-5301PC, Shimadzu); Gel imaging system (Bio-rad).

C. Experimental Method

1) Experiment grouping and contamination method. Divided the 20 Kunming mice into 4 groups, control group and 3 formaldehyde contamination groups, which was $0.5\text{ mg}/\text{m}^3$, $1.0\text{ mg}/\text{m}^3$ and $3.0\text{ mg}/\text{m}^3$. And there were five mice in each group.

2) Collection of bone marrow cells. After contamination, put the mice to death and get leg bone. Snip osteoepiphysis and expose pulp cavity. Then inject PBS and washout bone marrow into 1.5ml Eppendorf centrifuge tube. Centrifuge in 1500rpm for 5min and collect sediment. Wash the sediment twice with PBS. Keep it in 4°C.

3) Abstraction and purification of genome DNA in bone marrow. Centrifuge the collection of bone marrow at 1500rpm, 10min, 4 °C, discard supernatant, collect cell collection. Alligate the cells in TES(10mMTris-Cl, 1mM EDTA, pH8.0, 1%SDS), add Protease K to final concentration 50μg/ml, water bath in 55 °C for 2-3h until the admixture became clear. Then add equal volume of Phenol-Phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform respectively. Twice volume of precooling dehydrated alcohol to ethanol precipitation. Scrubbing with 70% ethanol. Airdry and dissolution in 100ulTE solution. Preserve in 4°C.

4) Assaying and purity test of DNA. Use the DNA Quant procedure to make DNA assaying according to UV absorption method [5]. And calculate the value of OD260: OD280. Then make electrophoresis to the DNA using 0.7% agarose gel. After that using gel-imaging system to analysis the purity of the electrophoresis results (Figure 1).

5) Random amplification polymorphism of genomic DNA. PCR reaction system (25μL). Genomic DNA template, 80ng. 10× reaction buffer (100mmol • L-1 Tris-HCl, pH 8.3, 15 mmol • L-1 MgCl₂, 500 mmol • L-1 KCl, 0.1 mmol • L-1 EDTA, 5 mmol • L-1 DTT, 50% glycerol, 0.1% Triton X 100), 2.5 μl. 200 μ mol • L-1 dNTPs (4 components are all 50 μ mol • L-1), 117 μ mol • L-1 random primers and 212 U TaqDNA polymerase. RAPD processes are as following. First of all, predegeneration for 5 min in 94°C. Secondly, run 35 cycles and each cycle contain steps as following. That is degeneration for 30s in 94°C, renaturation for 1min in 38°C, elongation for 1min in 72°C. Finally, incubation for 10min in 72°C. PCR product was preserved in 4°C before use.

6) Electrophoresis detection. RAPD products were separated by 1.4% agarose gel electrophoresis. EB staining. Observed the electrophoresis results and make photographic records on gel imaging system (Figure 2). Genomic template stability (GTS) can be calculated by GTS=(1-a/n) × 100% (a, RAPD polymorphism bands of the group exposed to formaldehyde. That is, compared with the control group, the total number of the new emerging and disappearing PCR bands in the group exposed to formaldehyde. n, the total number of PCR bands in control groups [6, 7]). Deal with the experimental data with origin7.0 statistical analysis software, and then carry out t test and charting.

TABLE I. LIST OF RAPD PRIMERS

Primer Sequence(5'→3')		Primer Sequence(5'→3')
1 CTG GCG AAC T		2 TCC GAT GCT G
3 CTG AGG TCT C		4 TCA TCC GAG G
5 ACC TTT GCG G		6 AAT GCC GGA G
7 CTG CGC TGG A		8 AAA GTG CGG C
9 AGA CCC AGA G		10 TCT CCG CCC T

III. RESULT

A. Analysis of genomic DNA purification results

DNA contents was determined by external absorbent method and calculated OD260:OD280=1.76 ~ 1.85. After electrophoresis image analysis system showed a single band, no trailing phenomenon (Figure 1). This showed that DNA extracted and purified by this method was high purity and not degradation. So we could get stable genome DNA randomly amplified product using such DNA as template for PCR amplification.

B. Effect of formaldehyde contamination on RAPD profiles

In the experiment, ten 10-mer priming oligonucleotides were used to analyze the PCR products and eight gave specific and stable result. In all cases, RAPD patterns generated by the formaldehyde-exposed plantlets were clearly different from those obtained using control DNA. The results obtained from four primers are presented (Fig. 2). And the data analysis of RAPD results is presented (Fig.3).

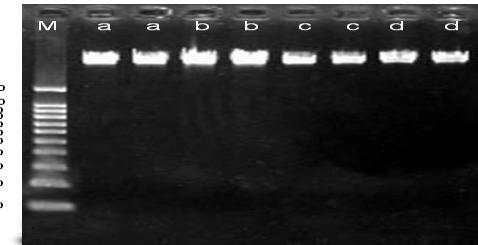
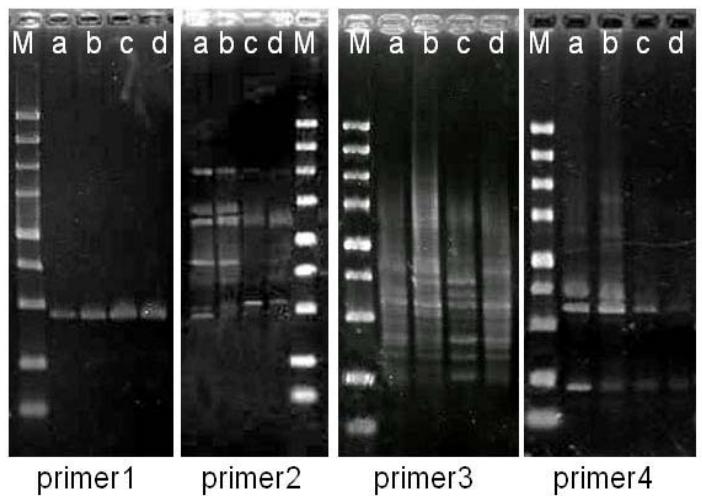


Figure 1. Electrophoresis patterns of bone marrow genome DNA (M: DNA marker, a: 0mg/m³, b: 0.5mg/m³, c: 1.0mg/m³, d: 3.0mg/m³)



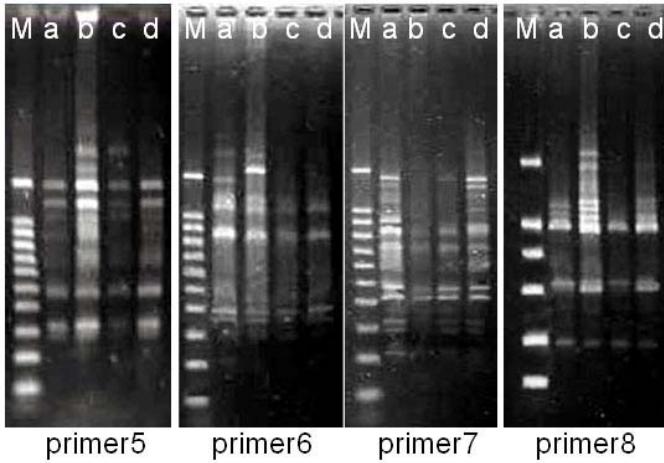


Figure 2. RAPD profiles of genomic DNA exposed to different formaldehyde concentrations

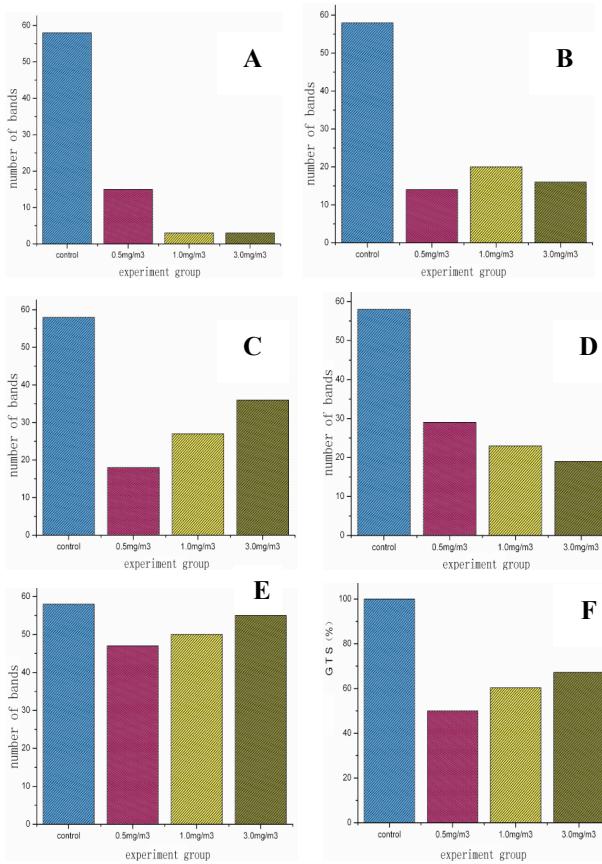


Figure 3. Data analysis of RAPD results

Note: A: figure of increased bands in three concentration group compared to control group, B: figure of disappeared bands in three concentration group compared to control group, C: figure of intensity changed bands in three concentration group compared to control group, D: figure of polymorphism bands in three concentration group compared to control group, E: figure of all changed band in three concentration group compared to control group, F: figure of GTS in three concentration group compared to control group

TABLE II. RAPD BANDS OF CONTROL GROUP AND EACH CONCENTRATION GROUP

primers	Concentration of formaldehyde (mg/m ³)											
	0.0	0.5	1.0	3.0	a	b	c	d	a	b	c	d
primer 1	1	0	0	1	0	0	0	1	0	0	0	0
primer 2	6	1	1	1	0	0	3	1	1	0	3	1
primer 3	10	6	1	2	1	2	0	6	0	0	2	3
primer 4	5	1	0	1	1	0	2	0	2	0	3	0
primer 5	8	2	0	3	0	1	2	0	4	1	0	3
primer 6	8	1	1	0	2	0	3	0	4	0	4	0
primer 7	15	0	11	0	3	0	8	0	5	0	4	4
primer 8	6	4	0	3	1	0	2	0	4	2	0	3
total bands	58	15	14	10	8	3	20	7	20	3	16	14
a+b					29				23			19
c+d						18			27			36
a+b+c+d							47		50			55

Note: a: increased bands, b: disappeared bands, c: fortified bands, d: weakened bands, a+b: polymorphism bands, c+d: intensity changed bands, a+b+c+d: all changed bands

IV. DISCUSSION

This experiment showed that the RAPD bands of control group were obviously different to the bands of other three-concentration group. And the number of changed amplification had positive correlation to the concentration of formaldehyde. With the concentration of formaldehyde increasing, the number of changed amplification bands rise. The analysis of GTS showed that with genome polymorphism degrading gradually, genome stability increased gradually. RAPD technology had successfully detected benzopyrene, heavy metals, radionuclides, estrogen and other pollutants could cause DNA damage and mutations of microorganisms, animals and plant cell in low-dose exposure [8,9]. Conte reported that fingerprinting technology such as RAPD, AFLP and RFLP could detect the temporary changes of DNA in biology cells, and this technology was more sensitive than conventional assay method of genetic toxicity [10]. This study indicated that the change between the concentration of formaldehyde and RAPD atlas showed a good dose - response relationship. It manifested the higher the concentration of formaldehyde, the more serious of DNA injury in liver cells of mice would be. The change of RAPD bands may be due to the change of initiation site of oligonucleotide caused by genome rearrangement. May also because of DNA damage induced by formaldehyde toxicity. This damage caused the changes in DNA conformation, and forms a new map of RAPD. But the mechanism needed further study [6, 11]. RAPD technology is a qualitative technique, and its reproducibility influence by many factors. So its stability was lower and needed further optimization for the conditions [12]. The strength of RAPD bands influenced by many factors such as the competition of PCR products, NA pollution, heterozygosity, comigration, and so on [13].

The results above signed that formaldehyde had significant genotoxic and damage effect to DNA of bone marrow in mice. Meanwhile, it verified that the change of DNA polymorphism obtained by RAPD technique could be a biomarker to detect genotoxic of formaldehyde.

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